Atopic Dermatitis: A Disease Caused by Innate Immune Defects?

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Atopic dermatitis (AD) is a common chronic inflammatory skin disease that has increased in prevalence over the last half century. A growing body of evidence suggests that there are a variety of defects in the innate immune system that collectively affect the development and severity of AD. The reduction in antimicrobial peptides, diminished recruitment of innate immune cells (PMNs, pDC, and NK cells) to the skin, epithelial barrier disruption, and TLR2 defects are just some of the credible explanations for AD patients' susceptibility to pathogens such as *Staphylococcus aureus*, herpes simplex virus, and vaccinia virus. Although the focus for several years has been to identify defects in the innate immune system that might explain AD patients' susceptibility to cutaneous pathogens, it has become clear that some innate immune defects might promote inflammation and thereby aggravate or even induce the development of AD. Here we review the innate immune system, and highlight many of the potential innate networks that may be important in AD patients susceptible to cutaneous pathogens.

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Editor's Note

Atopic dermatitis (AD) and other atopic diseases have been described throughout the history of medical literature. In ancient China, a clinical condition similar to what is now called atopy was described (Ring, 2005). Emperor Octavianus Augustus is one of the first individuals to be described with atopy, suffering, according to Suetonius, from ''extremely itchy skin, seasonal rhinitis and tightness of the skin'' (Suetonius: De Vita Caesarum; Ring, 2005). The term ''atopy'' was coined by Coca and Cooke (with the help of the linguist Edward D. Perry of Columbia University), who in 1923 attempted to develop a classification for ''hypersensitiveness'', an abnormal level of sensitiveness for which the mechanism was not known. ''Atopy'' is derived from the Greek ''ατοπία'', denoting a reaction that constituted a ''strange or eccentric disease'' (Coca and Cooke, 1923). While our understanding of atopy has advanced enormously since then, the detailed mechanisms of atopy and AD remain a mystery. In this issue, we begin a Perspectives series on AD in which De Benedetto *et al* describe the role of the innate immune response in AD, highlighting the ''out of place'' reaction that occurs. Attempts to understand the pathogenesis of atopy through animal models has proven difficult; in the second of the series Jin *et al* review animal models that have nonetheless lent us important clues. In future issues, articles related to the genetics of AD, its general immunology, and barrier function will be presented. Together, these contributions will update our readers on our understanding of the pathogenesis of atopy, that ''strange disease'' that Coca and Cooke first attempted to classify 85 years ago.

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Abbreviations: 1,25D3, 1,25(OH)$_2$vitamin-D$_3$; AD, atopic dermatitis; AMP, antimicrobial peptide; CE, cornified envelope; DC, dendritic cell; HBD, human β-defensin; HSV, herpes simplex virus; IL-1R, IL-1 receptor; KC, keratinocyte; KLK, kallikrein; LL-37, cathelicidin; LPS, lipopolysaccharide; MBL, mannose-binding lectin; MIP-3α (CCL20), macrophage inflammatory protein-3alpha; MyD88, myeloid differentiation factor 88; NK, natural killer; NOD, nucleotide-binding oligomerization domain; pDC, plasmacytoid DC; PGLYRP, peptidoglycan recognition proteins; PGN, peptidoglycan; PGRP, peptidoglycan recognition protein; PMN, neutrophil; PRR, pattern-recognition receptor; SC, stratum corneum; SPINK5, serine protease inhibitor, Kazal-type; TEWL, transepidermal water loss; TJ, tight junction; TLR, Toll-like receptor; VV, vaccinia virus

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INTRODUCTION

Atopic dermatitis

Atopic dermatitis (AD) is a highly pruritic, chronic inflammatory skin disease that affects up to 20% of children worldwide and can persist into adulthood (Leung *et al.*, 2004b). It has a significant impact on the quality-of-life of patients and their families and the economic impact is measured in billions of dollars (Akdis *et al.*, 2006; Delea *et al.*, 2007). More than 50% of patients develop asthma and other atopic disorders, adding further to the health and economic burden of this disease (Kapoor *et al.*, 2008). AD patients have frequent bacterial and viral skin infections (Nishijima *et al.*, 1995; Cho *et al.*, 2001). Approximately 80–100% of AD patients are colonized on nonlesional skin as compared to 5–30% of healthy controls (Hauser *et al.*, 1985; Breuer *et al.*, 2002). In addition to increased colonization, 50–60% of the *Staphylococcus aureus* found in AD patients are toxin producing (Akiyama *et al.*, 1996). Furthermore, AD patients can rapidly advance to superinfection with 10^7 organisms per cm^2 in acute lesions, as opposed to nonatopic controls who maintain a low bacterial burden (Leung and Bieber, 2003). AD patients with persistent *S. aureus* colonization despite therapy, are characterized by higher IgE levels suggesting that Th2 polarization adversely affects the immune response to this pathogen (Guzik *et al.*, 2005). About 30% of patients with AD report bacterial infections compared to only 6% of psoriasis patients (Christophers and Henseler, 1987). Cutaneous viral infections caused by vaccinia virus (VV) called eczema vaccinatum and by herpes simplex virus (HSV) referred to as eczema herpeticum have been shown to occur primarily in AD patients (Wollenberg *et al.*, 2003a). In fact it was in 1948, that the *Journal of Pediatrics* in a review article on generalized vaccinia recommended that ''patients with eczema should not be vaccinated and should not remain in the same household with those recently vaccinated'' (Fries and Borne, 1949). Since that time epidemiological data suggests that AD patients with more severe disease (earlier age of onset, persistence into adulthood, higher total IgE, higher Eczema Area and Severity Index scores) and with greater Th2 polarity (increased frequency of other atopic disorders and elevated serum thymus- and activation-regulated chemokine levels) are at greatest risk for skin infections with HSV or *S. aureus* (Wollenberg *et al.*, 2003b; Guzik *et al.*, 2005; Peng *et al.*, 2007; Beck *et al.*, 2008).

Over the last two decades it has become clear that the most effective mammalian response to microbes involves a delicate balance between the innate and adaptive arms of the immune system. Although the interactions between these two pathways are numerous and complex, the current data suggests that the susceptibility to cutaneous infections is largely due to abnormalities of the innate system (McGirt and Beck, 2006). Some of the innate immune defects observed in AD are primary defects such as epithelial barrier defects and defects in signaling or expression of innate receptors and others may be secondary to the effects of the adaptive immune response namely Th2 cytokines. For example, deficiencies in antimicrobial peptides (AMPs) and the barrier proteins observed in the skin of patients with AD may be due, in part, to the overexpression of Th2 cytokines such as IL-4 and IL-13 (Ong *et al.*, 2002; Howell *et al.*, 2007). After a brief introduction to the innate immune system in the section that follows, we will highlight many of the potential defects that may be important for AD patients' susceptibility to cutaneous pathogens.

Innate immune system

The immune system protects the host from pathogens and initiates the repair process following injury or trauma. In vertebrate animals this is achieved by a finely orchestrated interaction between the innate and adaptive immune pathways (Kabelitz and Medzhitov, 2007; Palm and Medzhitov, 2007). Phylogenetically, the innate immune system is the oldest and acts as the first line of defense against environmental insults. It acts rapidly, with remarkable ability to distinguish sequences unique to pathogens compared to self, but with less specificity for the individual pathogen. The innate immune system senses microbes through a group of germline-encoded proteins, named pattern-recognition receptors (PRRs; Janeway and Medzhitov, 2002). PRRs include transmembrane and intracellular receptors, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-leucine rich containing protein family such as NOD1 and 2, helicases such as retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5 and the double-stranded RNA binding kinase, as well as soluble molecules found in both intra- and extravascular compartments such as pentraxins (pentraxin-related protein and C-reactive protein), collectins (mannose-binding lectin (MBL) and ficolins; Table 1; Janeway and Medzhitov, 2002; Medzhitov, 2007). PRRs recognize highly conserved molecular patterns common to many classes of pathogens, known as pathogen-associated molecular patterns (Medzhitov and Janeway, 2002). Pathogen-associated molecular patterns include bacterial cell-wall components (such as lipopolysaccharide (LPS), peptidoglycan (PGN), and lipoteichoic acid), fungal cell wall (zymosan), viral double-stranded RNA molecules, and unmethylated CpG DNA primarily found in bacteria. PRR activation results in the production of cytokines, chemokines, and AMPs, as well as the activation and recruitment of immune cells (immature dendritic cells (DCs), natural killer (NK) cells, and neutrophils (PMNs)).

These innate responses occur rapidly and are efficient at killing pathogens and containing or limiting tissue injury. The innate immune system also initiates and determines the magnitude and the specific outcome of the adaptive immune response which takes days to develop, and provides long lasting immunologic memory (Hammad and Lambrecht, 2008). The adaptive immune response requires somatic mutations leading to the development of antigen-specific T-cell receptors (cell-mediated immunity) and immunoglobulins (humoral immunity).

Interestingly, recent studies have shown that adaptive immunity may in

Table 1. Pathogen related receptors (PRRs) that may be defective in subjects with AD

PRRs	Defects in AD	Cell	Ligands	Major function
TLRs	TLR2	KC, DC, LC, PMN, Monocyte, Mast cell, NK	Bacterial components (LPS, PGN, LTA) or yeast (Zymosan)	Production of AMPs, chemokines, and cytokines.
	TLR9	B cell, pDC, NK, KC	Viral and bacterial CpG	
NLRs	NOD1–2	KC, DC, LC, phagocytes,	PGN (Gram-positive and -negative bacteria)	Production of cytokines, chemokines, and AMPs.
CD14	?	DC, KC, macrophages	LPS and other bacterial components	Production of cytokines and chemokines
Soluble PRRs	MBL (?)		Surface of microbes	Opsonization or lysis of microbes. Leukocyte chemotaxis

AMP, antimicrobial peptide; DC, dendritic cell; KC, keratinocyte; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MBL, mannose-binding lectin; NK, natural killer; NLR, NOD leucine rich containing protein; NOD, nucleotide-binding oligomerization domain; pDC, plasmacytoid DC; PGN, peptidoglycan; PMN, neutrophil; TLR, Toll-like receptor.
?, contradictory evidence.

Table 2. Skin innate immunity and related defects observed in AD patients

Skin innate system	Major components	AD defects
Anatomical/ physical barrier	Cornified envelope	Reduced FLG, LOR, and INV expression; SPINK5 deficit; cystatin M/E deficit; enhanced SCCE expression; reduced lipids (ceramides, sphingosine); trauma from itch-scratch cycle
	Tight junctions	Reduced claudin-1
Cellular elements	PMN, NK, DC, LC, Mast cell, KC	Reduced function or migration into the skin of effector cells (PMN, NK, pDC); PRRs dysfunction (TLR2, TLR9, NOD1/2)
Secretory elements	AMP	Decreased AMPs (HBD2, HBD3, LL37, DCD, sphingosine)
	Cytokines/ chemokines	Reduction in MIP3α /CCL20, IL-8/CXCL8

AD, atopic dermatitis;AMP, antimicrobial peptide; DC, dendritic cell; DCD, dermcidin; HBD, human β-defensin; KC, keratinocyte; LL-37, cathelicidin; MIP-3α, macrophage inflammatory protein-3α; NK, natural killer; NOD, nucleotide-binding oligomerization domain; pDC, plasmacytoid DC; PMN, neutrophil; PRR, pattern-recognition receptor; SCCE, stratum corneum chymotryptic enzyme; SPINK5, serine protease inhibitor, Kazal-type; TLR, Toll-like receptor.

fact regulate the innate response and by so doing minimize the tissue damage that develops as a consequence of innate inflammatory cell influx. For example, regulatory T cells are able to repress the innate responses *in vitro* as well as *in vivo* (Lu *et al.*, 2006a; Ralainirina *et al.*, 2007). Interestingly, Lu *et al.*(2006a) have demonstrated that the protective actions of T_{reg} in an allograft tolerance model depend, at least in part, on the recruitment and activation of mast cells. Kim *et al.* have recently shown that nude mice (lacking T lymphocytes) inoculated with a sub-lethal dose of mouse hepatitis virus died from tissue damage caused by a ''cytokine storm'' (IFNγ and tumor necrosis factor-α) released by innate immune cells rather than overwhelming infection (Palm and Medzhitov, 2007; Kim *et al.*, 2007b). By adoptive transfer of specific lymphocyte subsets in Rag-deficient mice, which lack both T and B lymphocytes, they were able to show that this overly robust innate immune response could be attenuated by CD4+ or CD8+ lymphocytes (for example, cells of adaptive immune system). Although it has generally been accepted that the adaptive response arises several days after the innate, these studies suggest that part of the adaptive response may in fact coincide with the early innate immune response and provide a critical suppressive action on the inflammation induced by innate pathways. Whether the adaptive response (for example, Th2 cytokines) is suppressing the innate immune response in AD patients is a plausible theory.

The skin and mucosal surfaces (gastrointestinal, respiratory, urogenital) by virtue of their direct interface with the environment are the sites where innate and adaptive immunity are established and have their greatest interaction (Clark and Kupper, 2005). The skin innate immune system consists of three major components: anatomical/physical barrier (stratum corneum (SC) and intercellular junctions), cellular (antigen presenting cells, keratinocytes (KCs), mast cells, and PMNs), and secretory elements (AMPs, cytokines, and chemokines; Table 2). We will review the components of a healthy cutaneous immune response and highlight which of these are altered in patients with AD.

PATTERN RECOGNITION RECEPTORS

TLR1–10 are the best characterized human PRRs and are expressed on both the cell surface (TLR1, 2, 4–6, 10) and intracellularly in the endosomes (TLR3, 7–9; Kaisho and Akira, 2006). The function of TLRs is to induce inflammation and DC maturation, which determines the character and quality of the adaptive immune response. Most TLR ligands promote the development of Th1 or Th17 cells that are important for antibacterial and antiviral immunity (Weaver *et al.*, 2007). Interestingly, weak TLR2 and TLR4 signals in the context of allergen exposure in the skin

and lung, respectively, have been shown to promote a Th2-biased immune response (Chisholm *et al.*, 2004; Eisenbarth *et al.*, 2004). Therefore, the character of the innate immune response may actually cause inflammatory diseases such as AD by both promoting a Th2 response to otherwise innocuous environmental antigens (for example, allergens) as well as preventing the effective eradication of *S. aureus*.

The expression and function of most TLRs was initially characterized on antigen-presenting cells, PMNs, and mast cells. More recently, KCs have been recognized as active participants in the innate immune response in the skin (Esche *et al.*, 2004). KCs constitutively express mRNA for TLR1, 2, 3, 4, and 5, and potentially 6–10 (Meyer *et al.*, 2003; Kollisch *et al.*, 2005; McInturff *et al.*, 2005). The functions of TLR 1–5, 7, and 9 have been implied by the expression of immune response genes following TLR-specific ligand stimulation (Meyer *et al.*, 2003; McInturff *et al.*, 2005; Lebre *et al.*, 2007). In response to ligation of these receptors, KC produce a number of mediators relevant for an immediate response to pathogens such as epithelial adhesion molecules, and molecules involved in direct antimicrobial actions, cell activation, apoptosis, proliferation, and chemotaxis.

When compared to most other TLRs, TLR2 recognizes a remarkably broad range of ''pathogen-associated motifs'' or pathogen-associated molecular patterns, such as several components of Gram-positive bacteria (that is, PGN and lipoteichoic acid) as well as LPS from various Gram-negative bacteria and fungi and potentially even herpes viruses (Sato *et al.*, 2006; Zahringer *et al.*, 2008). HSV can trigger responses through TLR2 (TLR3, TLR7, and TLR9; Lund *et al.*, 2003; Sato *et al.*, 2006). Importantly, a recent study highlights the association of several TLR2 polymorphisms and increased viral shedding and increased genital lesion counts in patients with genital herpes (HSV-2; Bochud *et al.*, 2007). It is appealing to hypothesize that TLR2 pathway defects may be responsible for AD patients' susceptibility to *S. aureus* and HSV.

TLR2's broad microbial responsiveness probably comes from its unique ability to homodimerize as well as heterodimerize with TLRs 1 and 6 (Triantafilou *et al.*, 2006). An impairment of TLR2-mediated inflammatory cytokine production (IL-1β and tumor necrosis factor-α) was recently demonstrated in peripheral blood monocytes from AD patients stimulated with the synthetic TLR2 ligand, $C_{81}H_{156}N_{10}O_{13}S$ 3HCl (Hasannejad *et al.*, 2007). We have noted a similar defect in KC propagated from nonlesional skin of AD patients (McGirt *et al.*, 2006). This finding in both monocytes and KC could not be explained by differences in TLR2 surface expression (Hasannejad *et al.*, 2007). Interestingly, this impairment was specific to TLR2, with no defects noted in response to TLR4 ligands. It is important to note that the ''weak'' TLR2 response observed in AD KC and monocytes may not only render AD patients incapable of eradicating the bacteria colonizing their skin but may also promote a Th2 response as noted above. We have recently demonstrated that another function of TLR2 on KC is to enhance/repair tight junction (TJ) function (Beck, De Benedetto personal communication). Whether this important function is also defective in AD KC is an important question.

The TLR that has been evaluated most extensively for its role in the development and infectious complications observed in patients with AD is TLR2. Humans heterozygous for the TLR2 R753Q mutation are prone to staphylococcal infections (Lorenz *et al.*, 2000). In one study 11.5% of AD patients were heterozygous for this missense mutation and this tracked with a more severe disease phenotype (Ahmad-Nejad *et al.*, 2004). This same research group demonstrated that monocytes from AD patients heterozygous for this TLR2 mutation had dramatically reduced IL-8 production in response to PGN compared to wild-type AD patients (Mrabet-Dahbi *et al.*, 2008). This work contrasts with a study of 275 German parent-offspring trios, which utilized four common TLR2 haplotypes and found no association with AD (Akdis *et al.*, 2006). In summary, this work suggests that the TLR2 pathway may be defective in AD patients on a genetic or acquired basis with possibilities that include altered TLR2 structure or altered expression/function of signaling proteins or negative regulatory elements that have recently been implicated in attenuating TLR pathways. Potential negative regulatory pathways include proteins such as Toll-interacting protein or soluble TLRs (Liew *et al.*, 2005). A preliminary screen of 50 AD patients for variations in the coding region of Toll-interacting protein identified two unique amino-acid substitutions in exons 4 and 6 but these were not associated with AD in a larger cohort (>300 patients; Schimming *et al.*, 2007).

Most TLRs and IL-1 receptor (IL-1R) family members transduce a signal through the intracellular adapter molecule called myeloid differentiation factor 88 (MyD88), which leads to the nuclear translocation of NF-κB and other transcription factors (Brikos and O'Neill, 2008). In contrast, TLR3 utilizes a MyD88-independent pathway that leads to type 1 IFN production, which is thought to be critical for viral clearance. Interestingly, in a murine *S. aureus* cutaneous infection model, MyD88- and IL-1R-deficient mice had much higher bacterial counts and more reduced tissue neutrophilia than the TLR2-deficient mouse suggesting that these innate pathways must diverge, and highlighting the importance of IL-1R and other MyD88-dependent pathways in containment of *S. aureus* skin infections (Miller *et al.*, 2006). Importantly, reconstitution of IL-1R-deficient mice with wild-type bone marrow failed to correct the defect suggesting that resident cells (not hematopoietic cells) are the ones that release the PMN chemoattractants in response to IL-1. The role of IL-1 superfamily members in AD remains unclear (Braddock *et al.*, 2004). In one study there was an increased ratio of IL-1R antagonist to IL-1α in the SC of AD patients, which was similar to that observed in patients with psoriasis and greater than that seen in healthy controls (Terui *et al.*, 1998). This is in contrast to a study demonstrating greater IL-1 release in peripheral blood mononuclear cells and purified monocytes from AD pa-

tients in response to LPS compared to controls (Thestrup-Pedersen *et al.*, 1990). Another member of the IL-1 family, IL-18, known to be a mediator of inflammation and innate immunity, is expressed by KC and like IL-1β is downregulated by corticotropin-releasing hormone (Park *et al.*, 2005). As AD is known to be exacerbated by stress, it can be hypothesized that stress-induced release of corticotropin-releasing hormone and the ensuing reduction of IL-18 and IL-1β may also be invoved in AD patients' susceptibility to cutaneous infections.

Although TLR3 binds viral double-stranded RNA, its role in host defense against the DNA viruses, HSV and vaccinia, remain quite perplexing. Two unrelated children were recently reported with dominant negative TLR3 allele who developed HSV-1 encephalitis with no skin disease (Zhang *et al.*, 2007). These patients' NK and CD8+ T cells had impaired responsiveness to the TLR3 ligand (poly(I:C)), whereas their blood derived DCs and KC responded normally suggesting that TLR3 is essential for primary immunity to HSV-1 in the central nervous system but not at other anatomical sites such as the skin (Zhang *et al.*, 2007). Interestingly plasmacytoid DCs, which are factories for the potent antiviral type 1 IFNs are not thought to express TLR3 (Iwasaki and Medzhitov, 2004). TLR3−/− mice infected intranasally with vaccinia had improved survival, reduced lung inflammation suggesting that TLR3 signaling contributes to the pathogenesis of severe poxvirus infections (Hutchens *et al.*, 2008). There have been no published studies on TLR3 polymorphisms in AD but one might expect that any observed mutation would most likely lead to a gain-in-function rather than a loss if it was to explain these patients susceptibility to eczema vaccinatum or eczema herpeticum.

TLR9, which is found within the endosome, can bind both viral and bacterial CpG DNA and therefore may be a relevant PRR for both *S. aureus* and HSV or vaccinia infections. It is expressed on plasmacytoid DCs, NK cells, B cells, and KC. Activation of TLR9 on plasmacytoid DCs (pDCs) and B cells induce a Th1-biased response. In a recent publication, the TLR9 polymorphism C-1237T, which results in higher promoter activity, was associated with the intrinsic variant of AD (Novak *et al.*, 2007). Of all the AD cases, 10-20% are called intrinsic based on the lack of associated atopic disorders and no allergen sensitizations. But intrinsic cases are otherwise clinically indistinguishable from the more common extrinsic variant with the exception that intrinsic subjects have slightly reduced expression of Th2 cytokines (IL-5 and IL-13) in lesional skin. One would assume that such a gain-in-function TLR9 mutation would potentially be protective against numerous microbes.

CD14 is a multifunctional receptor for LPS and other bacterial wall components (Koppelman and Postma, 2003). As CD14 has also been found to induce cellular activation in response to lipoteichoic acid through a TLR2-dependent pathway, (Schroder *et al.*, 2003) and has binding affinity for PGN (Dziarski, 2003), it is thought to be important in host response to *S. aureus*. Similar to TLR2 signaling, CD14 utilizes MyD88 to activate NF-κB. It is also shown to induce IL-1β production through a caspase-1-dependent pathway (Tschopp *et al.*, 2003). Although it is expressed as a soluble or membrane-bound receptor predominantly on monocytes, it has also been found on a variety of cells, including KC (Song *et al.*, 2002).

Fueled by the ''Hygiene Hypothesis'' and the epidemiological data showing that the presence of LPS is inversely correlated with atopy, numerous genetic studies of atopic populations have been performed looking at CD14 variants (Koppelman and Postma, 2003; Sengler *et al.*, 2003; Liang *et al.*, 2006). These studies have shown conflicting results with different atopic phenotypes, using different methodologies. Nevertheless, the consensus seems to be that although CD14 is a polymorphic gene, no polymorphisms appear to strongly associate with the atopic phenotypes evaluated to date. Equally confusing is the identification of both reduced and/or elevated levels of soluble CD14 in breast milk from mothers with at-risk children (Jones *et al.*, 2002; Zdolsek and Jenmalm, 2004; Rothenbacher *et al.*, 2005). The general assumption is that elevated levels of soluble CD14 indicate a recent or ongoing infection with either a Gram-positive or -negative bacteria. Therefore lower levels would reflect a reduced capacity to respond to microbial signals or decreased exposure to microbial signals. We have not observed any differences in the expression of CD14 on KC propagated from nonlesional skin of AD, psoriasis, or nonatopic controls (McGirt *et al.*, 2006). In conclusion, the current data does not clearly implicate a role for CD14 in the pathogenesis of AD or its susceptibility to cutaneous infections.

NOD1 (or CARD4) and NOD2 (or CARD15) receptors make up the CARD subfamily within the larger NOD-leucine rich repeat protein family, a group of intracellular innate immune receptors that respond to a variety of microbial products. The other NOD-leucine rich containing protein subfamily is the pyrin subfamily, which includes Nalp1–14 (Wilmanski *et al.*, 2008). The best known of these is Nalp3 or cryopyrin. Activating mutations in this protein have been linked to a number of autoinflammatory diseases such as familial cold autoinflammatory syndrome, Muckle–Wells syndrome and neonatal onset multisystem inflammatory disease (Farasat *et al.*, 2008). NOD-leucine rich containing protein family members make up the inflammasome, a macromolecular structure so named for its ability to induce caspase-1 activation and ultimately the release of IL-1β and IL-18 (Drenth and van der Meer, 2006). Little is known about the microbial specificity of the pyrin subfamily members although both Nalp1 and 3 are thought to respond to *S. aureus* (Mariathasan *et al.*, 2006; Wilmanski *et al.*, 2008). More is known about NOD1 and NOD2, which respond to degradation products of PGN (Girardin and Philpott, 2004). Specifically, NOD1 senses diaminopimelic acid-type PGN, which is produced by Gram-negative bacteria, and NOD2 senses muramyl dipeptide, a motif found in PGNs from all bacteria, including *S. aureus* (Girardin

and Philpott, 2004). Recently, KCs were shown to express NOD1 and NOD2, which were presumed to be functional as stimulations with PGN resulted in IL-6 production (Song *et al.*, 2002). Furthermore, KC stimulated with the NOD2-specific ligand, muramyl dipeptide, produced AMP, human β-defensin (HBD) 2 (Voss *et al.*, 2006; Kim *et al.*, 2008b). Importantly, NOD1 is located on a region of chromosome 7p14-p15 that has been linked with atopy (Weidinger *et al.*, 2005). Polymorphisms in NOD1 (Weidinger *et al.*, 2005), NOD2 (Kabesch *et al.*, 2003; Macaluso *et al.*, 2007), and Nalp12 (Macaluso *et al.*, 2007) have been associated with the phenotype of AD or allergy.

Peptidoglycan recognition proteins (PGLYRPs) are innate immunity molecules that are secreted and were first identified in insects (Mathur *et al.*, 2004; Lu *et al.*, 2006b). Mammals have four PGLYRPs. PGLYRP-1, -3, and -4 are able to kill bacteria by associating with the PGN on bacterial cell walls (Dziarski and Gupta, 2006). This is a different mechanism than that used by AMPs that kill bacteria by membrane permeabilization. Interestingly, PGLYRP-2 is an amidase and has anti-inflammatory actions as it hydrolyzes bacterial PGN and therefore reduces its proinflammatory actions through other innate networks. PGLYRP-1 is expressed primarily in the granules of PMNs. PGLYRP-2 is secreted from the liver into the blood (Dziarski and Gupta, 2006). PGLYRP-3 (or PGRP-1α) and PGLYRP-4 (or PGRP-1β) are released from epithelial cells of the skin, eyes, mouth, and intestinal tract and are bactericidal for many Gram-positive and -negative bacteria (Lu *et al.*, 2006b). Expression of PGLYRP-3 is induced in primary human KC by stimulation with the staphylococcal-specific pathogen-associated molecular patterns, lipoteichoic acid (McGirt *et al.*, 2006). Interestingly, PGLYRP-3 and PGLYRP-4 genes are located in the epidermal differentiation gene cluster on chromosome 1 within the PSOR4 and ATOD2 loci (Sun *et al.*, 2006). Clearly further studies are needed to characterize the importance of these receptors in recognition of *S. aureus* in patients with AD.

Other important components of the innate immune system are the soluble PRRs such as collectins, ficolins, and pentraxins. They recognize unique motifs on bacteria (Gram-positive and -negative), fungi, and virus. Soluble PRRs are thought to act as opsonins or they can directly activate the complement system (Lu *et al.*, 2002). MBL, a collectin family member and ficolins (L-ficolin and H-ficolin) initiate the lectin pathway of complement activation on binding to microbial carbohydrates (Holmskov *et al.*, 2003; Endo *et al.*, 2006), whereas pentraxins activate the classical complement pathway (Bottazzi *et al.*, 2006). Complement activation promotes the opsonization of microbes and direct killing of pathogens through the formation of the membrane attack complex, the cytolytic end product of the complement cascade. Membrane attack complex is known to form a transmembrane channel, which causes osmotic lysis of the target cell. Complement activation induces the release of proteolytic fragments of C3 and C5, which have potent chemotactic activity for innate immune cells (Carroll and Fischer, 1997; Medzhitov, 2007). MBL-deficient mice are more susceptible to intravenous inoculation with *S. aureus* than wild-type mice (Shi *et al.*, 2004; Kars *et al.*, 2005). Interestingly, serum MBL deficiency has been observed in 10–15% of Caucasians with significantly higher percentages reported in subjects of African or South American Indian descent (Bouwman *et al.*, 2006). Such deficiencies and their variant alleles within the coding region of MBL have been associated with increased susceptibility to bacterial infections in neutropenic patients, poorer prognosis in cystic fibrosis patients, or more severe meningococcal disease (Eisen and Minchinton, 2003; Bouwman *et al.*, 2006; Kaur *et al.*, 2006b). There is very preliminary epidemiologic evidence to suggest MBL is important for clearance of several common viruses (hepatitis B, HIV, and influenza A) but virtually nothing is known about how MBL levels might affect the immune response to HSV infection (Bouwman *et al.*, 2006). We recently looked at MBL levels and a functional assay for MBL (for example, determining MBL C4b deposition capacity with an anti-human C4 monoclonal antibody; IBT Reference Laboratory, Lenexa, KS) and found no difference in AD patients who had never had an episode of eczema herpeticum and patients who had this HSV skin complication (Wollenberg, A and Beck, LA, personal communication).

Despite studies showing a correlation between MBL levels or complement activity and peripheral eosinophilia or FEV1 in patients with asthma and allergic rhinitis, the majority of papers have failed to show an association of any atopic phenotype and frequency of MBL polymorphisms (Aittoniemi *et al.*, 2005; Leung *et al.*, 2006; Kaur *et al.*, 2006a; Muller *et al.*, 2007; Wang *et al.*, 2007). Nevertheless, a recent report highlights a clear association between extremely low MBL levels and the BB MBL haplotype in several members of a Turkish family who also suffered from recurrent staphylococcal infections (skin, ear, and airway) and a pruritic, eczematous dermatitis (Brandrup *et al.*, 1999). Small genetic studies targeting AD patients have shown conflicting results with lack of an association between MBL2 polymorphisms and reduced MBL levels in Japanese subjects (Hashimoto *et al.*, 2005), whereas a report on >150 Brazilian AD patients demonstrated that this variant was observed more frequently than in a healthy control population with an OR of 2.4 (Brandao *et al.*, 2008). Further studies are needed to sort out the role of these soluble PRRs in AD and its susceptibility to cutaneous colonization and infection.

INNATE IMMUNE CELLS

Natural killer cells

NK cells are an important component to the innate immune system that lyse host cells which have been infected with microbes without any need for prior activation. The lysis is mediated by the release of perforin and granzyme from cytoplasmic granules. In addition, NK cells can release numerous inflammatory cytokines, which likely recruit

other innate immune cells. Circulating NK cells are significantly reduced in AD patients and are functionally defective as noted by the reduced release of a Th1 cytokine, IFNγ but normal levels of the Th2 cytokine, IL-4, and increased apoptosis (Katsuta *et al.*, 2006). These functional defects were reversed when the activated peripheral blood monocytes were removed. This finding needs to be confirmed and its relationship to cutaneous infections explored.

Plasmacytoid dendritic cells

pDCs are a critical source for the antiviral type I IFNs (IFNα and IFNβ). Although the number of pDCs in the circulation is increased in AD (Uchida *et al.*, 2001), skin lesions have significantly diminished numbers compared to other inflammatory skin conditions such as psoriasis, contact dermatitis, or lupus (Wollenberg *et al.*, 2002). pDCs from AD patients may also be functionally impaired as cross-linking of their high affinity IgE receptor (FceR1) reduces IFN production (Novak *et al.*, 2004).

Neutrophils

A striking finding in lesional biopsies from AD patients is the absence of PMNs, even in the setting of intense scratching or *S. aureus* colonization and/or infection. A number of studies have pointed to a chemotactic defect in AD PMNs (Michaelsson, 1973) and such defects were found to correlate with markers of AD disease severity–IgE levels (Hill *et al.*, 1974) and recurrent bacterial infections (Rogge and Hanifin, 1976; Dahl *et al.*, 1978; Ternowitz *et al.*, 1987). PMN functional activities are particularly impaired in AD patients with concomitant bacterial infections especially during the course of an infectious episode (Ternowitz *et al.*, 1987). Rogge and Hanifin (1976) showed impaired PMN chemotactic activity in patients with severe erythroderma and *S. aureus* colonization assessed by the Boyden chamber. Other groups suggested decreased chemotactic responses to be a separate defect in AD without correlation to infection or IgE levels (Snyderman *et al.*, 1977; Galli *et al.*, 1983). Hanifin *et al.* suggested that factors in the plasma of AD patients might be responsible for the decreased responsiveness of AD PMNs to specific chemoattractants (Rogge and Hanifin, 1976). Other functional alterations observed in AD PMNs included an impaired release of β-glucuronidase (Christophers and Henseler, 1987), defects in LTB4 production and release (Schafer *et al.*, 1991), absent deposition of extracellular PMN granule proteins (lactoferrin and PMN elastase) in skin biopsies with normal serum elastase levels (Ott *et al.*, 1994), and impaired phagocytosis and a reduced capacity to produce reactive oxygen species (Mrowietz *et al.*, 1988). AD PMNs do not seem to have baseline adherence differences when compared to controls although they demonstrated a blunted response to histamine and ispoproterenol-induced downregulation of adhesion (Thulin *et al.*, 1980). We found that PMNs from AD patients had a markedly decreased CD11b-upregulation response to both activating stimuli (CXCL8/IL-8 and CXCL1/GRO-a) and priming (GM-CSF) stimuli (Bankova *et al.*, 2007). The work of numerous laboratories suggests that the b2 integrins, Mac-1 (CD11b/CD18), and LFA-1 (CD11b/CD18) involved in a very different way in PMN migration that are essentially organ specific. Both components of Mac-1—CD11b and CD18—are critical to PMN migration to the skin. Therefore, the diminished upregulation of CD11b, would explain the lack of PMNs in AD skin but other organs such as the lung, joints, and peritoneum would be able to compensate for this Mac-1 defect by engaging CD18-independent or CD11b-independent mechanisms for PMN recruitment. The lack of PMNs may also be due to the reduced tissue neutrophilia which is due to the reduced production of PMN chemoattractants such as the cathelicidin (LL-37), which acts through the FMLP receptor or reduced expression of IL-8 (CXCL8; Nomura *et al.*, 2003; Howell *et al.*, 2006c). As PMNs are critical cells in the initial response to all pathogens it is not surprising that a defect in PMN recruitment to the skin would make AD patients susceptible to a wide range of cutaneous microbes.

ANTIMICROBIAL PEPTIDES

An important component of the cutaneous innate immune response is the production of AMPs. KCs produce several peptides with antimicrobial actions including S100 proteins, ribonuclease 7, LL-37, human defensin-α and -β, sphingosine, and dermcidin (Schroder and Harder, 2006). Several chemokines, such as macrophage inflammatory protein-3α (MIP-3α (CCL20)), monokine induced by IFN-γ (CXCL9), IFN-inducibe protein (CXCL10), and IFN-inducible T-cell α-chemoattractant (CXCL11), have also been shown to have antimicrobial activity (Yang *et al.*, 2003). AMPs directly kill a broad spectrum of microbes including Gram-positive and -negative bacteria as well as fungi and certain viruses. The antimicrobial properties of these peptides arise from their ability to integrate into and disrupt the cellular membrane of the offending organism (Izadpanah and Gallo, 2005). AMPs can also modulate host immune response including leukocyte chemotaxis and activation of PRRs (Izadpanah and Gallo, 2005). With the exception of HBD1, most AMPs are undetectable in skin under basal conditions, but are induced after injury or inflammatory stimuli (Liu *et al.*, 2002; Sorensen *et al.*, 2005; Schroder and Harder, 2006; Aberg *et al.*, 2008). LL-37, HBD2, and HBD3 have been shown to have antistaphylococcal activity (Schibli *et al.*, 2002; Menzies and Kenoyer, 2005). LL-37 is also recognized for its antiviral activity against HSV-1, HSV-2, and VV (Howell *et al.*, 2004, 2006b). In KCs, HBD2 and LL-37 are stored in lamellar bodies along with barrier lipids and cornified envelope (CE) proteins (Oren *et al.*, 2003; Braff *et al.*, 2005). The colocalization of AMPs and CE proteins suggests that these two innate functions may interact in some way.

The antimicrobial activity of LL-37 is controlled at both the transcriptional and post-transcriptional level. Infection, inflammation, wounding, and 1,25-dihydroxyvitamin D3 are known to induce LL-37 gene expression (Schauber *et al.*, 2006). But the inactive precursor (hCAP18) must be enzymaticly processed to release the C-term-

inal peptide (that is, LL-37), which confers it with antimicrobial properties. The hCAP18 N-terminal peptide is a cathelin-like protein with weak antimicrobial as well as antiproteinase activity. It has been suggested that this protein may protect cells from excessive proteolysis by host or microbial cysteine proteases (Zaiou *et al.*, 2003). A recent study by Yamasaki *et al.* (2006), has demonstrated that the activation of hCAP18 in KC is regulated by the serine proteases, SC tryptic enzyme (kallikrein 5; KLK5) and SC chymotryptic protease (KLK7). Interestingly, the SPINK5-deficient mouse, which lacks the serine protease inhibitor lympho-epithelial Kazal-type-related inhibitor, has increased epidermal antimicrobial activity, which can be normalized with immunoabsorption of LL-37 (Yamasaki *et al.*, 2006). These observations suggest that the proteolytic activity at the skin surface can modulate the actions of AMPs.

Unfortunately microbes such as *S. aureus* produce proteases or toxins, which can interfere with host AMPs. For example, aureolysin, a metalloproteinase produced by *S. aureus*, can inactivate LL-37 (Sieprawska-Lupa *et al.*, 2004). Several *S. aureus* strains have been identified that express the gene, *mprF*, which confers resistance to several host defense peptides such as defensins and protegrins by modifying the charge on bacterial membranes (Peschel *et al.*, 2001).

Besides their antimicrobial property, the AMPs act as a link between innate and adaptive immune responses. LL-37 and some defensins have been shown to be chemoattractant for PMNs, monocytes, and T cells (Yang *et al.*, 2001; Niyonsaba *et al.*, 2004). β-Defensins exhibit chemotactic activity for immature DC by binding to the CC chemokine receptor CCR6 (Yang *et al.*, 1999). LL-37 is also involved in wound repair by promoting angiogenesis and epithelial growth (Carretero *et al.*, 2008). Recently, Aberg *et al.* (2008) have shown that mice deficient in CRAMP (the murine homolog of LL-37) have a delay in the permeability barrier recovery after a wound injury.

Ong *et al.* (2002) were the first to recognize that AD patients had reduced HBD2 epidermal immunoreactivity and mRNA expression compared to psoriasis patients. A follow-up study by Nomura *et al.* (2003) found a reduction in HBD2, as well as HBD3 in lesional skin biopsies from AD compared to psoriasis patients using GeneChip microarrays. The reduced AMP expression was due, in part, to the inhibitory effects of the Th2 cytokines (IL-4 and IL-13) and the immunomodulatory cytokine, IL-10 on KCs (Ong *et al.*, 2002; Nomura *et al.*, 2003; Howell *et al.*, 2006b). Several studies have shown that the AMP, LL-37 is necessary for an adequate response to both HSV and VV (Howell *et al.*, 2004, 2006b) and that LL-37 levels from skin biopsies are significantly reduced in patients with AD compared to psoriasis (Ong *et al.*, 2002). Indeed, Howell *et al.* report that the LL-37-deficient (Cnlp−/−) mouse skin has higher replication of HSV than wild-type mice, suggesting that the lack of this AMP may provide an explanation for AD patients' predisposition to eczema herpeticum (Howell *et al.*, 2006c). This reduced production of LL-37 may also predispose AD patients to eczema vaccinatum (Howell *et al.*, 2006a). In addition, work by Kim *et al.* (2007a), recently demonstrates lower level of MIP-3α in AD compare to psoriasis, likely due to the overexpression of Th2 cytokines. Interestingly, the authors show the importance of MIP-3α in the innate immune response against VV.

Sphingosine is a metabolite of ceramide produced by the outer layers of the skin that has antimicrobial actions on *S. aureus* at physiological levels, and is thought to be important in preventing bacterial colonization on healthy skin. The SC of AD patients have significantly reduced levels of sphingosine compared to controls, which is assumed to be the consequence of altered ceramide metabolism. This may be involved in AD patients' high colonization rate with *S. aureus* (Arikawa *et al.*, 2002).

Dermcidin (DCD) is a recently discovered broad-spectrum AMP, which is produced in human eccrine glands, and secreted in sweat (Rieg *et al.*, 2005). AD patients have significantly reduced levels (Rieg *et al.*, 2005). AD patients with the greatest reduction in dermcidin in the sweat had the greatest problems with bacterial and viral skin infections (Rieg *et al.*, 2005). We can infer from these data that the lack of dermcidin in the sweat of AD patients has a notable role in contributing to the high susceptibility of the patients to skin colonization and infection (Rieg *et al.*, 2005).

Vitamin D has recently attracted considerable attention, at least in part, for its ability to regulate AMP expression in monocytes and KCs (Gombart *et al.*, 2005; Weber *et al.*, 2005). KCs are the only cells in the body able to synthesize the active metabolite $1,25(OH)_2$vitamin-D_3 (1,25D3) from its inactive precursor, 7-dehydrocholesterol. The key enzymes for this conversion are D-25 hydroxylase (the equivalent of CYP27 in the liver) and 25OHD-1αhydroxylase (the equivalent of CYP27B1 in the kidney; Bikle *et al.*, 2004). 1,25D3 mediates its effects by binding to the vitamin D receptor, a member of the nuclear hormone receptor family of Zinc finger transcription factors. Previous studies have shown that 1,25D3 induces KC to differentiate as determined by the expression of involucrin and transglutaminase, and the formation of CE. Bikle *et al.* (2004) showed that mice deficient in 25OHD-1αhydroxylase had a defect in cornified layer and a delayed recovery in permeability barrier function after acute disruption of the SC and a perturbation of the normal calcium gradient. These findings suggest that vitamin D is important in epidermal differentiation and barrier.

More recently, vitamin D has been recognized for its antimicrobial actions. This was first suggested when the vitamin D response element was identified in the promoter region of the human LL-37 gene (Gombart *et al.*, 2005). Stimulation of KC with 1,25D3 not only increased expression of the inactive precursor (hCAP-18) but also increased the mature peptide (LL-37) and enhanced the antimicrobial activity against *S. aureus*, suggesting that 1,25D3 can increase LL-37 transcrip-

tion and activation (Schauber *et al.*, 2006). Liu et al. (2006) showed that stimulation of TLR2 on monocytes by *Mycobacterium tuberculosis* increases expression of vitamin D related genes (that is, 25OHD-1αhydroxylase and vitamin D receptor), leading to an increased killing of this intracellular bacteria. Based on these findings, the authors have speculated that African-Americans' susceptibility to *M. tuberculosis* infection may be due to low serum vitamin D level that are thought to be due to decreased UV penetration as a consequence of their higher epidermal melanin content. These authors showed that vitamin D supplements given to African-American subjects boosted monocyte LL-37 levels to the level observed in Caucasians. 1,25D3 is also important for the innate immune response to injury. KCs surrounding a wound, demonstrate increased expression for LL-37, TLR2, and CD14 all of which are induced by 1,25D3 (Schauber *et al.*, 2007). Interestingly, the authors also reported an increase *in vivo* of LL-37 and TLR2 following application of topical 1,25D3 in healthy volunteers. Studies in inflammatory bowel disease suggest that vitamin D is important in mucosal barrier homeostasis by preserving the integrity of TJs (Kong *et al.*, 2007). Although the mechanism underlying these observations is still unclear, a similar biology may be observed in the skin. These findings highlight the importance of vitamin D in microbial recognition and response during skin infection or just injury.

Interestingly, vitamin D receptor polymorphisms have been described in several inflammatory diseases such as psoriasis (Park *et al.*, 1999), diabetes (Gyorffy *et al.*, 2002), Crohn's disease (Simmons *et al.*, 2000), and asthma and atopy (Raby *et al.*, 2004).

EPITHELIAL BARRIER

There is little doubt that there is an epidermal barrier defect in AD. The evidence to support this comes from the following findings: enhanced transepidermal water loss (TEWL), reduced irritancy threshold, increased percutaneous absorption, and dry appearance of lesional skin (Madison, 2003). The first physiological evidence of an impaired barrier function was the demonstration of increased TEWL (Werner and Lindberg, 1985). The extent of barrier dysfunction correlates with the degree of inflammation within AD lesions (Lebwohl and Herrmann, 2005) and AD severity in general (Barker *et al.*, 2007; Hon *et al.*, 2008). It is important to recognize that TEWL reflects barrier function from inside-out, whereas the more clinically relevant direction is outside-in as this is the direction taken by allergens, irritants, colonizing bacteria, and pollutants. Many groups are working on methods to more accurately assess barrier function from this direction both *ex vivo* and *in vivo*.

The epidermis also functions as a primary defense and biosensor to the external environment. Some of this barrier function resides within the SC, but once this barrier has been breached the TJs found at the level of the stratum granulosum are the next level of defense. A disturbance in barrier favors the penetration of microbes and allergens and other environmental insults (toxins, irritants, pollutants) and is now recognized as a central feature of AD (Cork *et al.*, 2006). SC has been likened to a brick wall, consisting of terminally differentiated KCs or corneocytes (bricks), which are surrounded by a matrix of specialized lipids (mortar; Elias and Feingold, 1992). The major lipids in SC are ceramides (50% by mass), fatty acids (10–20% by mass), and cholesterol (25% by mass). This creates a barrier that helps to keep water within the body and prevent the entrance of pathogens and allergens (Choi and Maibach, 2005). AD patients have reduced levels of the SC lipids, ceramide (Imokawa, 2001; Pilgram *et al.*, 2001). Stress may aggravate this by the production of endogenous glucocorticoids, which suppress epidermal lipid production (Choi *et al.*, 2005). Lastly, a hallmark of AD is intense pruritus, which, characteristically occurs before a skin lesion develops. This intense itch leads invariably to extensive scratching, and this mechanical trauma is also capable of disrupting the CE. In addition to providing a barrier, CE also inhibits pathogen colonization by virtue of its low water content, acidic pH, resident microflora and production of numerous AMPs (Elias and Steinhoff, 2008).

AD barrier dysfunction may also have a genetic basis, which was first suspected when genome-wide studies identified linkage to the epidermal differentiation complex on Ch 1q21 (ATOD2; Cookson *et al.*, 2001). In 2006, null mutations in a specific gene within the epidermal differentiation complex, namely, filaggrin (*FLG)* were identified and shown to be strongly linked to the phenotype of AD and asthma associated AD, whereas no associations were observed with psoriasis, another inflammatory skin disease with epidermal differentiation complex (1q21-PSOR4) linkage (Palmer *et al.*, 2006; Zhao *et al.*, 2007). Although up to 30% of AD patients from European cohorts have been found to have null mutations in *FLG*, it seems unlikely that this will explain the increase in TEWL that is observed in nearly 100% of AD patients with active disease (Palmer *et al.*, 2006; Hubiche *et al.*, 2007; Gupta *et al.*, 2008). We and others have shown that FLG is not expressed at other mucosal surfaces relevant for atopic diseases (upper or lower airway or esophagus), suggesting that the association of *FLG* mutations with other atopic disorders is likely due to the common feature of allergen sensitization through the skin (Ying *et al.*, 2006; Morar *et al.*, 2007; De Benedetto *et al.*, 2008b). FLG levels in the skin can also be modulated by Th2 cytokines with IL-4 and IL-13 downregulating expression on human differentiated KC (Howell *et al.*, 2007). Although the number of *FLG* mutations identified is now over 12 it is still not known whether and how these translate into quantifiable measures of skin barrier dysfunctions (Chen *et al.*, 2008; Nomura *et al.*, 2008). We and others have identified several other CE proteins found within the epidermal differentiation complex (Ch 1q21) that are reduced in expression profiling studies of AD skin or epithelial explants and include loricrin, involucrin, and late CE proteins suggesting there are other proteins that are important in the

barrier function of this outer layer of the skin (Sugiura *et al.*, 2005; Morar *et al.*, 2006; De Benedetto *et al.*, 2007; Kim *et al.*, 2008a).

Premature desquamation can also diminish barrier function at the level of the SC (Cork *et al.*, 2006). Numerous proteases have been identified that disrupt corneodesmosomes, a structure important for CE. The balance between the levels of proteases (chymotryptic, tryptic, serine, and cysteine proteases) and protease inhibitors (serine protease inhibitor Kazal-type 5 (SPINK5)] and the cysteine protease inhibitor (cystatin M/E)) may determine whether an individual has excessive corneodesmosome breakdown and thinning of the SC (barrier) or too little corneodesmosome breakdown and thickened SC (barrier; Cork *et al.*, 2006). Mutations in SPINK5 are thought to cause the profound skin barrier dysfunction and atopic diathesis characteristic of Netherton's syndrome (Chavanas *et al.*, 2000). Ultrastrutural analyses reveal an increased cleavage of corneodesmosomes and reduction in intercorneocyte cohesion (Cork *et al.*, 2006). Over the past decade, several groups have demonstrated a dysregulation of epidermal genes encoding for other proteases or antiproteases in patients with AD including dipeptidyl peptidase 10 (*DPP10*), *SPINK5* (Nishio *et al.*, 2003), transglutaminase (*TGM*), and SC chymotryptic enzyme (*SCCE, KLK7*; Vasilopoulos *et al.*, 2004).

Intercellular junctions have long been recognized as the regulators of permeability in simple epithelium. Intercellular junctions consist of adherens and TJs, which are closely associated ultrastructurally because of their association with a circumferential belt of actin. TJ are typically observed on the apical aspects of stratum granulosum cells and appear as ''kissing points'' in electron microscopy images where the intercellular space is almost obliterated and hence their alternative name, zonulae occludens (Schluter *et al.*, 2004; Niessen, 2007). It was not until 2002 that their role in stratified squamous epithelium of the skin was appreciated (Pummi *et al.*, 2001). Furuse *et al.* (2002) reported that claudin-1-deficient mice died within 24 hours of birth with wrinkled skin, severe dehydration, and increased epidermal permeability as measured by dye permeability and TEWL. Importantly, these mice had a normal functioning SC (and normal expression of SC proteins) but a dysfunctional TJ.

TJ constitute the ''gate'' to the passage of ions and molecules through the paracellular pathway and function as a ''fence'' within the plasma membrane to create and maintain apical and basolateral membrane domains or cell polarity (Schluter *et al.*, 2007). They consist of a multiprotein complex. The TJ proteins identified so far include the transmembrane proteins; claudin family members, junctional adhesion molecule family members, occludin, and tricellin and the cytoplasmic plaque proteins to which these transmembrane proteins attach; zonulae occludens (ZO)-1, ZO-2, ZO-3, MUPP-1, MAGI, and cingulin (Niessen, 2007). The cytoplasmic plaque proteins bind to actin and myosin and are thought to communicate changes in junctional integrity through Rho-specific and other signaling pathways (Aijaz *et al.*, 2005). This suggests that the loss of barrier integrity would initiate a signaling response that among other functions would induce KC proliferation. We have recently shown that claudin-1 expression is markedly reduced in AD skin and this suggests that the barrier defect in this disease may also be at the level of TJ (De Benedetto *et al.*, 2008a). The import of claudin-1 in TJ formation was highlighted in a seminal paper demonstrating effective TJ formation in fibroblasts when they were reconstituted with claudin-1 (and not occludin) and that the TJ formed by claudin-1 had strands noted by TEM that were continuous (Furuse *et al.*, 1998). In addition a recently described syndrome, which is due to claudin-1 mutations called neonatal ichthyosis with sclerosing cholangitis further highlights the biological significance of this protein. These subjects develop some of the features observed in AD, such as erythema, dry flaky skin, and alopecia in addition to unique features such as severe liver and gallbladder abnormalities (Hadj-Rabia *et al.*, 2004).

It is well recognized that microbes present multiple virulence factors capable of interfering with the host barrier function. Various strains of *S. aureus* produce virulence factors with proteolytic activity (Kanzaki *et al.*, 1997; Miedzobrodzki *et al.*, 2002). Three major extracellular proteinases have been described and include metalloproteinases (*S. aureus* metalloproteinase; aureolysin), serine proteinases (SASP; V8 protease, glutamyl endopeptidase), and the cysteine (thiol) proteinases (staphylopain; Shaw *et al.*, 2004). How these enzymes contribute to the skin barrier impairment in AD is still unclear. Staphylococcal toxins especially the exfoliative toxins A, B, and D, are glutamate-specific serine proteases that cleave a single peptide bond in the extracellular region of desmoglein 1, causing the loss of KC desmosomal adhesion in the superficial epidermis (Hanakawa *et al.*, 2002; Nishifuji *et al.*, 2008). On the other hand, certain viruses and bacteria use intercellular junction proteins as receptors to infect KC. For example, nectin-1, an immunoglobulin-like molecule which colocalizes with E-cadherin to form adherens junctions in epithelial cells, has been recognized as a receptor for HSV-1 (Spear and Longnecker, 2003). Importantly, nectin-1 is not available for viral binding unless TJ are disrupted. Calcium depletion of human epithelial cell lines *in vitro,* which disrupts TJ, rapidly induced redistribution of nectin-1 to the cell surface, and increased viral attachment (Galen *et al.*, 2006).

It is not just microbes that have evolved to express factors capable of breaking barrier but also common ubiquitous allergens such as house dust mites (*Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f); Chapman *et al.*, 2007). These allergens like many others encode for both cysteine and serine proteases. The mechanisms by which house dust mite proteases activate target cells are beginning to be defined. Two general mechanisms have emerged, which are not mutually exclusive. First, Der p 1 can cleave several relevant cell surface molecules including CD23, CD25, and CD40 (Shakib *et al.*, 1998; Ghaemmaghami

et al., 2002). Second, Der p can activate the protease-activated receptor family of G-protein coupled cell surface receptors (Kauffman *et al.*, 2006), which has been shown to induce airway inflammation by stimulating the release of cytokines and chemokines from respiratory epithelial cells (Kauffman *et al.*, 2006). Although studies on the effect of dust mite protease in human skin are still limited, evidence is growing that protease-activated receptor-2 is important in epidermal permeability barrier homeostasis by mediating signaling from serine proteases in the SC (Hachem *et al.*, 2006).

Altogether, these findings suggest that patients with AD may have a skin barrier defect that has both a genetic and acquired basis. This defect may be further exacerbated by environmental factors such as scratching, use of detergents, microbial colonization/infection, and exposure to protease-bearing allergens. The assumption is that this barrier defect leads to greater microbial invasion and allergen sensitization.

EFFECT OF TH2 CYTOKINES ON CUTANEOUS INNATE IMMUNE RESPONSES

Substantial evidence supports the idea that allergic diseases have a ''Th2 bias'', with excess production of Th2 (IL-4, IL-5, and IL-13) cytokines that recapitulate many of the key features including IgE isotype switching and eosinophilia. The precise molecular mechanisms for the ''Th2 bias'' in atopy are complex, and involve both genetic and environmental factors (Leung *et al.*, 2004a).

Leung *et al.* have implicated the Th2-polarized environment as a key factor in the epithelial production of fibronectin and fibrinogen which can act as substrates for *S. aureus* adherence (Cho *et al.*, 2001). Growing evidence suggests that this Th2 bias may also adversely affect the innate immune response in the skin of AD patients (Howell, 2007). In a recent collaborative study with Dr Howell and Dr Leung, we have noted that most AD patients have reduced FLG immunoreactivity in lesions compared to nonlesional skin and this deficiency is due, in part, to the overexpression of Th2 cytokines, which we showed could downregulate FLG expression in differentiated KC (Howell *et al.*, 2007). This work suggests that filaggrin defects can develop as an acquired and/or genetic defect. Two other CE proteins, loricrin and involucrin, appear to be reduced in the lesional skin of AD patients. Like filaggrin the expression of these proteins were also downregulated by Th2 cytokines (Kim *et al.*, 2008a).

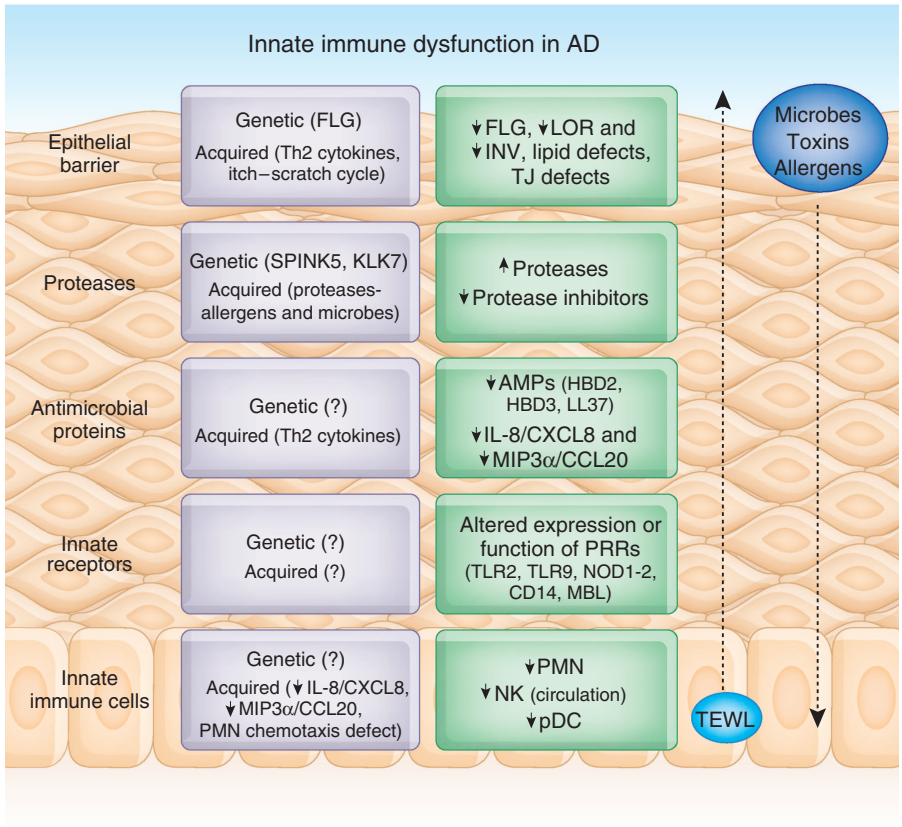

Figure 1. Overview of innate immune defects observed in AD. A variety of defects both genetic and acquired have been identified in the innate immune system in AD and include barrier defects, reduced antimicrobial peptide release, genetic polymorphisms, and dysfunction in PRRs, and diminished recruitment of innate immune cells (PMNs, pDC, and NK cells) to the skin. Some of these defects precede the development of the disease and others develop as a consequence of the disease process and affect AD severity. The skin barrier function is impaired in AD as a consequence of reduced lipids (sphingosine and ceramide), abnormal keratinization which is due to dysfunctional filaggrin and other CE components and mechanical trauma or scratching. Clinically this is supported by the increased transepidermal water loss (TEWL) observed in both lesional and nonlesional skin. This barrier breakdown creates a portal of entry for pathogens, allergens and toxins. Additionally, AD keratinocytes have an aberrant response to microbes that in addition to the diminished recruitment of innate immune cells (PMNs, pDC, and NK cells) to the skin may account for AD patients' susceptibility to pathogens such as *S. aureus*, HSV, and VV. The reduced recruitment of cells of the innate immune system may be explained in part by polymorphisms in pathogen related receptors such as TLR2, TLR9, NOD1 or 2, and possibly CD14 or MBL. The production of antimicrobial peptides (LL-37, HBD2, HBD3, Dermcidin and CCL20/MIP3α) is reduced in AD patients compared to either healthy controls or psoriasis. This is thought to be due, in part, to the Th2 cytokines produced by inflammatory cells, which have an inhibitory effect on keratinocyte production of these peptides and on CE proteins. The paucity of tissue PMN in AD lesions may be explained in part by reduced chemoattractants such as LL-37 and CXCL8/IL-8, but also appears to be due to an inherent defect in circulating PMNs that results in reduced upregulation of the β2 integrin, CD11b, which is important for skin migration. Circulating NK cells are significantly reduced in AD patients and are functionally defective as noted by the reduced release of the Th1 cytokine, IFNγ Lastly, AD skin lesions have significantly diminished numbers of pDC compared to other inflammatory skin. pDCs are a critical source for the antiviral type I IFNs (IFNα and IFNβ).

Moreover, recent studies have highlighted the negative effect of Th2 cytokines on the lipid components of the CE. Kurahashi *et al*. (2008) demonstrated in an animal model that the exogenous application of IL-4 delays the recovery of skin barrier after both tape stripping as well as acetone disruption. IL-4 has also been shown to inhibit ceramide synthesis in cultured KCs (Hatano *et al*., 2005; Elias and Steinhoff, 2008). Interestingly, Kobayashi *et al*. (2004) reported that IL-4 treatment of cultured KC monlayers enhanced the permeability of these cells to FITC-dextran which was dose-dependent fashion. In summary, this data suggests that Th2 cytokines adversely affect barrier function of the CE. Remarkably little is known about Th2 effects on TJ function.

IL-4 induces IgE production in B cells and suppresses anti-infectious immune responses by downregulating AMPs and inhibiting Th1 immunity (Biedermann, 2006) It has been demonstrated by Nomura *et al*. that the cytokine milieu in AD prevents the induction of multiple innate immune response genes which is thought to be due to lower levels of proinflammatory cytokines (such as tumor necrosis factor-α or IFNγ) and increased Th2 cytokines (Nomura *et al*., 2003). Expression profiling studies on AD skin samples have revealed reduced levels of IL-8 (CXCL8), induced nitric oxide synthetase, HBD2 and 3, and hCAP transcripts when compared to psoriasis (Nomura *et al*., 2003; Howell *et al*., 2006a). This same group showed that Th2 cytokines (IL-4 and IL-13) inhibit the production of AMPs (HBD2 and 3) and the antimicrobial chemokine MIP-3α (CCL20), which is also important for the recruitment of immature DCs (Nomura *et al*., 2003; Kim *et al*., 2007a). The KC production of induced nitric oxide synthetase (Paludan *et al*., 1999) and tumor necrosis factor-α-induced IL-8 are inhibited by IL-4 (Raingeaud and Pierre, 2005). IL-8 is a potent chemokine that attracts PMNs into the skin where they phagocytize and kill bacteria; whereas induced nitric oxide synthetase kills microbes by producing nitric oxide. Interestingly, L-4 transgenic mice develop pruritic, inflammatory skin lesions that are similar to those observed in humans with AD confirming that local skin expression of Th2 cytokines are likely responsible for at least some of the features observed in AD (Leung *et al*., 2004a).

CONCLUSION

It is clear that there are a variety of defects in the innate immune system, ranging from barrier defects to reduced AMP release to genetic polymorphisms in PRRs, that collectively affect the development and severity of AD (Figure 1). Some of these defects precede the development of the disease and others develop as a consequence of the disease process. Although the focus for several years has been to identify defects in the innate immune system that might explain AD patients' susceptibility to cutaneous pathogens, it has become clear that some innate immune defects (for example, TLR9 gain-in-function polymorphisms) might promote inflammation and in so doing induce the development of AD. Certainly the reduction in AMPs, diminished recruitment of innate immune cells (PMNs, pDC and NK cells) to the skin, epithelial barrier disruption and TLR2 defects are just some of the credible explanations for AD patients' susceptibility to pathogens such as *S. aureus*, HSV, and VV. How current topical and systemic therapies (both OTC and Rx) affect these key innate pathways is still largely unchartered territory. We are just scratching the surface of the skins' defense system but it promises to be an exciting ride with many more insights to be made.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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